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Pseudocrepidobothrium ludovici sp. n. (Eucestoda: Proteocephalidea), a parasite of *Phractocephalus hemioliopterus* (Pisces: Pimelodidae) from Brazilian Amazon

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***Pseudocrepidobothrium ludovici* sp. n. (Eucestoda: Proteocephalidea), a parasite of *Phractocephalus hemioliopterus* (Pisces: Pimelodidae) from Brazilian Amazon.** - *Pseudocrepidobothrium ludovici* sp. n., is described from the intestine of the pimelodid fish, *Phractocephalus hemioliopterus* (Bloch and Schneider, 1801) (Pisces: Pimelodidae), from the Amazon River in Brazil. The new species differs from the only other known congeneric species *Pseudocrepidobothrium eirasi* (Rego & de Chambrier, 1995), by the absence of ventral lateral posterior appendix on each side of the proglottides. Furthermore, the new species differs from *P. eirasi* by several other morphological characters such as the number of testes (21-51, x = 32 versus 37-79, x = 55), the absence of polar structure in the eggs, the structure of the scolex and the disposition of vitelline follicles. Prevalence of infection with *Pseudocrepidobothrium ludovici* is 12/29 (41%) in Brazil. No *P. ludovici* was found in Peru into the 11 *P. hemioliopterus* studied. A total of 12228 proteocephalidean cestodes were found in a single host specimen: 10641 *Pseudocrepidobothrium eirasi* (Rego & de Chambrier, 1995) (87 % of counted tapeworms), 1100 *P. ludovici* sp. n. (9%), 383 *Scholzia emarginata* (Diesing, 1850), 84 *Chambriella* sp., 15 *Proteocephalus hemioliopteri* de Chambrier & Vaucher, 1997, 4 *Zygobothrium megacephalum* Diesing, 1850, and 1 *Ephedrocephalus microcephalus* Diesing, 1850. *Pseudocrepidobothrium ludovici* sp. n. represents the seventh proteocephalidean species found in *Phractocephalus hemioliopterus*.

Keywords: Taxonomy - morphology - Pisces - South America - new species - tapeworm - distribution - guild.

INTRODUCTION

Among the six proteocephalidean species (Cestoda) found in the pimelodid catfish *Phractocephalus hemioliopterus* (Bloch & Schneider, 1801), one was classified provisionally into the genus *Crepidobothrium* Monticelli, 1900 as *Crepidobothrium eirasi* Rego & de Chambrier, 1995 (Rego and de Chambrier, 1995). Based on distinct scolex suckers and morphology of reproductive organs, this species was later allocated to a new genus, *Pseudocrepidobothrium* (see Rego & Ivanov, 2001). Another unknown proteocephalidean tapeworm belonging to *Pseudocrepidobothrium* Rego & Ivanov,

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2001 was discovered in the intestine of *P. hemioliopterus* during a survey of fish parasites conducted in Brazil between 1992 and 1995 by Amilcar Arandas Rego and the junior author. Since this cestode differs from the only other known species of *Pseudocrepidobothrium*, it is described here as a new taxon.

MATERIALS AND METHODS

Twenty-nine specimens of *Phractocephalus hemioliopterus* (Bloch & Schneider, 1801) (vernacular name in Brazil "Pirarara") were caught by local fishermen at Itacoatiara, Amazon River, about 200 km east of Manaus, Brazil, on 15-25 October 1992 (8 specimens) and 1-21 November 1995 (21 specimens). Eleven specimens of *P. hemioliopterus* were also obtained from the Iquitos market at Loreto, Peru, on 23 May 2005 (2 specimens), on 9-19 September 2006 (2 specimens), on 12-20 October 2009 (4 specimens), and on 4-12 October 2011 (3 specimens).

The fish were examined for internal parasites immediately after their capture. The parasites were isolated from the host intestine and fixed with hot 4% neutral formaldehyde solution and subsequently stored in 70% ethanol. These specimens were then stained with Mayer's hydrochloric carmine solution, dehydrated in an ethanol series, cleared with eugenol (clove oil) and mounted in Canada balsam. For histology, fragments of strobila were embedded in paraffin wax, transversely sectioned at 15-18 µm intervals, stained with Weigert's hematoxylin and counterstained with 1% eosin B with one drop of acetic acid/100 ml solution (Scholz & Hanzelová, 1998; de Chambrier, 2001; Oros *et al.*, 2010). Eggs were examined in distilled water. Three specimens were used for scanning electron microscope observations using the procedure outlined by de Chambrier *et al.* (2008). Furthermore, numerous additional specimens of the new *Pseudocrepidobothrium* were found among the Woodland material (slides and spirit material) deposited at the Natural History Museum, London (NHMUK) 1964.12.15.71-86, 1965.2.23.156-158, 1983.5.17.2-10 and 1965.2.23.146-155, ("Cotype" of *Myzophorus pirarara*), mixed with *M. pirarara* [= *Scholzia emarginata* (Diesing, 1850)].

The specimens described hereafter are deposited in the helminthological collection of the Natural History Museum, Geneva, Switzerland (MHNG), the Natural History Museum London (NHMUK), the Institute of Parasitology, Ceské Budejovice (IPCAS) and the Helminthological collection of the Museo Argentino de Ciencias Naturales, Buenos Aires (MACN). All measurements are given in micrometers unless otherwise indicated. Abbreviations used in descriptions are as follows: x = mean; n = number of measurements; CV = coefficient of variation.

RESULTS

Pseudocrepidobothrium ludovici sp. n. Figs 1-16

TYPE MATERIAL: Holotype MHNG INVE 22003, 1 whole mounted slide, field number Br 334. – 29 paratypes MHNG INVE 22000, 22108, 30531-32, 79281-85, 79302, 79306-20, 79327, 79335, 79340-41. – 2 paratypes IPCAS C-610, field number Br 785, Br 649 3/5z; 1 paratype NHMUK 2012.1.23.1, field number Br 785.

OTHER MATERIAL: From Itacoatiara, Amazon River, Amazonas Province, Brazil; collected 15-17.09.1992; MHNG INVE 22001, 22003, 22016, 79281-85, 79305. – Same locality as in

previous series, collected 01-18.10.1995: MHNG INVE 22000, 22047, 22103, 22108, 25600, 25610, 27437, 28298, 30531-32, 31199, 35186, 79302-04, 79306-20, 79327, 79333, 79335, 79337-42, 79345, 79349-50, 79354, 79356, 79360, 79363-64, 79388; NHMUK 2012.1.12.1; IPCAS No C-610 (Br 695z paratypes, cross section; Br 649 3/5 & Br 804, Br 804a), MACN No. 520/1-3 (Br 649 3/5y).

TYPE LOCALITY: Itacoatiara, Amazon River, Amazonas Province, Brazil, 17.09.1992. 03.1536°S 58.4382°W, Field number Br 334, A. de Chambrier & A. A. Rego leg.

DESCRIPTION (BASED ON 32 ENTIRE SPECIMENS): Proteocephalidae, Proteocephalinae. Small-sized worm, 7-23 mm long, up to 1150 wide, flattened dorsoventrally. Strobila acraspedote, anapolytic, bearing 20-36 proglottides in total, 9-22 immature, 1-6 mature, 3-19 gravid. Proliferation zone posterior to scolex short, up to 620 long and 283-765 wide. Immature and mature proglottides wider than long; pregravid proglottides wider than long, then longer than wide and gravid proglottides longer than wide. Some abnormal proglottides (e.g. with hypertrophy of vitelline follicles) were not considered in this study.

Scolex massive, round, 515-1020 in diameter (x = 775, n = 23) (Figs 1-4, 7), clearly separated from strobila. Apical tegumental folds present (Figs 2, 4). Four heart-shaped suckers, with notched posterior margin, disposed dorsally and ventrally by pairs, 230-385 (x = 290; n = 12) in diameter (Fig. 5). Apical organ absent. Scolex usually rectangle-shaped in apical view. Surface of scolex uniformly covered with capilliform filitriches (Fig. 6).

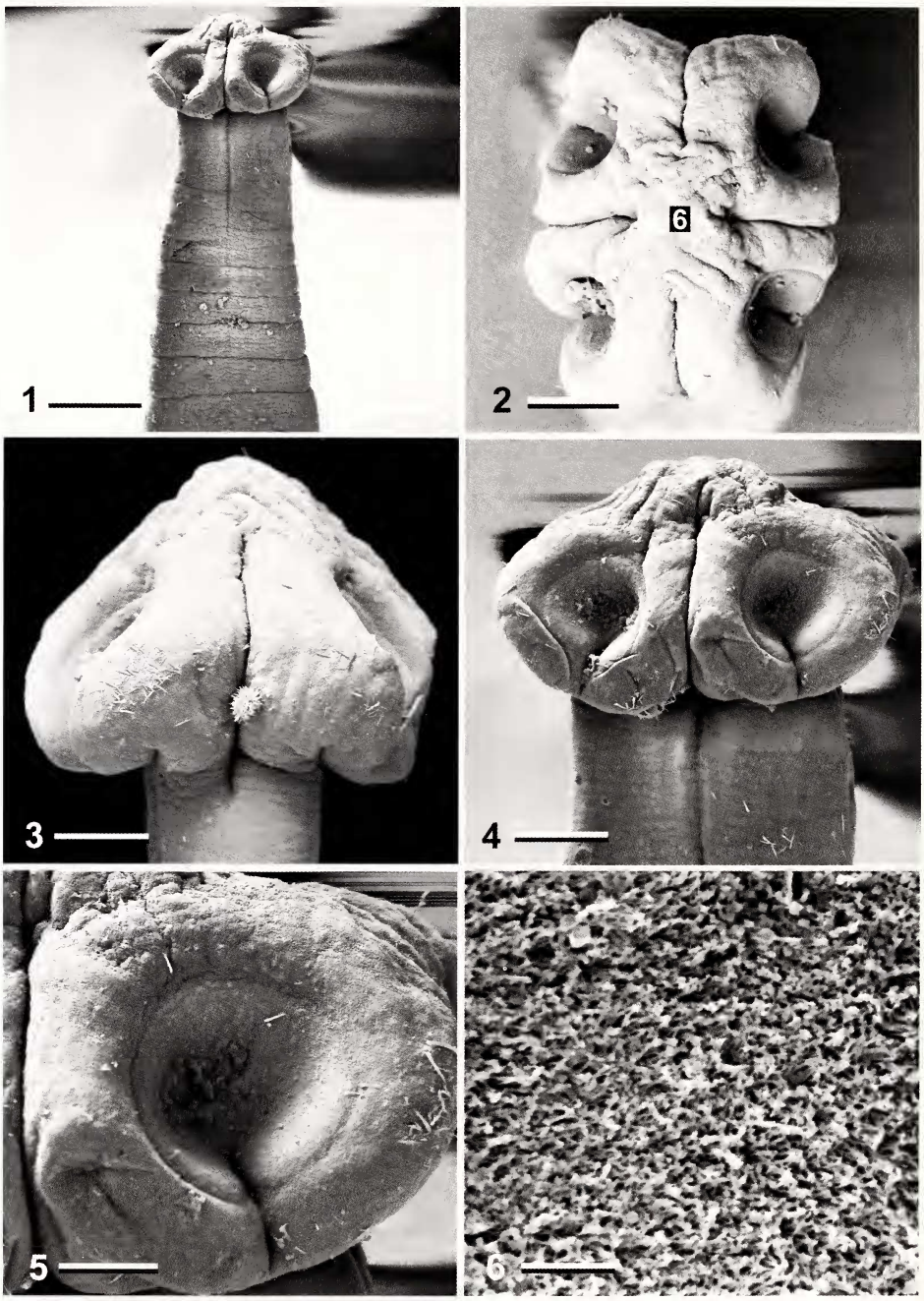
Internal longitudinal musculature weakly developed (Figs 13, 14) forming small anastomosed bundles of muscular fibers. Osmoregulatory canals usually situated between vitellaria and testes. Ventral canals about 35 in diameter with a secondary canal situated posteriorly near lateral margin and which seems to end at ventral surface (Fig. 8). Dorsal osmoregulatory canals about 15 in diameter, sometimes anastomosed or double.

Testes medullary, spherical to oval, 50-95 by 25-85 in diameter, numbering 37-79 (x = 55, n = 37, CV = 19%), in one (rarely two) layer, in two lateral fields (Figs 8-10, 13), usually connected with some testes anteriorly; testes degenerated in last gravid proglottides. Vas deferens coiled, very thin-walled, reaching to midline of proglottis, rarely overlapping it (Fig. 8). Cirrus-sac elongate to piriform, thin-walled, 155-260 long and 65-125 wide, representing 25-35% (x = 30%, n = 30, CV = 9%) of proglottis width. Cirrus occupying 35-57% (n = 26) of cirrus-sac length (Fig. 11).

Genital ducts passing between osmoregulatory canals. Genital atrium present. Genital pores irregularly alternating, situated at 15-29% (x = 22%, n = 28, CV = 14%) of proglottis length.

Vagina posterior (in 53% of proglottides) or anterior (in 47% of proglottides, n = 112) to cirrus-sac, in proximal part lined with chromophil cells. Muscular terminal sphincter present (Fig. 11). Mehlis' glands 35-100 in diameter, representing 6-14% of proglottis width.

Ovary medullary, bilobed, butterfly-shaped in gravid proglottides, 310-565 wide, occupying 53-67% (x = 59%, n = 30, CV = 6%) of proglottis width (Figs 8-10, 14).



FIGS 1-6. *Pseudocrepidobothrium ludovici* sp. n. from *Phractocephalus hemioliopterus*. Scanning electron photomicrographs. MHNG INVE 79302, paratype. (1) Scolex, dorsoventral view, with a anterior part of strobila. (2) Scolex, apical view. (3) Scolex, lateral view. (4) Scolex, dorsoventral view. (5) Detail of a sucker. (6) Capilliform filitriches near the center of the apical region. Scale-bars: 1 = 300 µm; 2 = 130 µm; 3 = 95 µm; 4 = 110 µm; 5 = 60 µm; 6 = 3 µm.

Vitelline follicles medullary and paramuscular (according to de Chambrier, 1990), oval to elongate, small, in two lateral fields, absent in preporal area, occupying porally 63-78% (x = 69%; n = 16) and aporally 75-95% (x = 84%; n = 17) (Figs 8-10, 13, 14).

Anlage of uterus medullary, already present in immature proglottides. Uterus with 14-20 very short lateral diverticula on each side (Fig. 12). Formation of uterus of type 1 according to de Chambrier et al. (2004a): uterine stem with tubular concentration of numerous intensely staining cells and with lumen in last immature and first mature proglottides (Figs 8, 9, 13, 14). In mature proglottides, thin-walled lateral diverticula appear. In pregravid proglottides, eggs filling uterine stem and diverticula. In gravid proglottides, uterus sometimes opening precociously ventrally by one longitudinal aperture and sometimes conserving eggs up to last proglottis. In last proglottides, uterus occupies up to 71% of proglottis width (Fig. 11)

Eggs spherical, with thin, hyaline outer envelope, up to 60 in diameter; inner envelope consisting in two-layered embryophore, with external thick layer, 17 in diameter, and nucleate irregular envelope, 12-14 in diameter; oncospheres 8-9 in diameter, with 3 pairs of embryonic hooks, 5-6 long (Figs 15, 16).

TYPE-HOST: *Phractocephalus hemioliopterus* (Bloch & Schneider, 1801), (Siluriformes: Pimelodidae).

SITE OF INFECTION: From anterior to middle of the intestine.

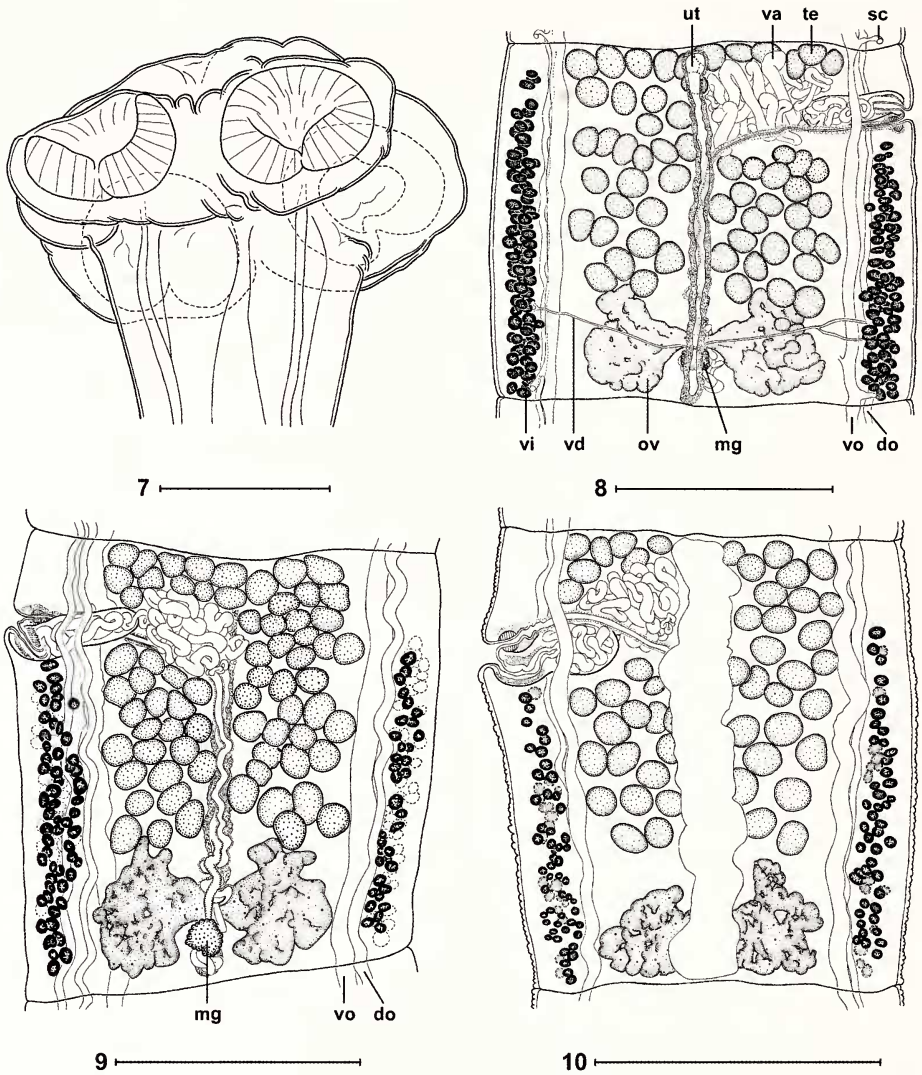
PREVALENCE: 12/29 (41%) in Brazil, 0/11 (0%) in Peru.

ETYMOLOGY: The new species is named in honour of Ludovic Ruedi, brother of the first author.

DIFFERENTIAL DIAGNOSIS: The present species is placed in *Pseudocrepidobothrium* Rego and Ivanov, 2001 (Proteocephalinae) because of the medullary position of the genital organs, the medullary and paramuscular position of vitellaria and the heart-shaped structure of suckers (Freze, 1965; Schmidt, 1986; Rego and Ivanov, 2001).

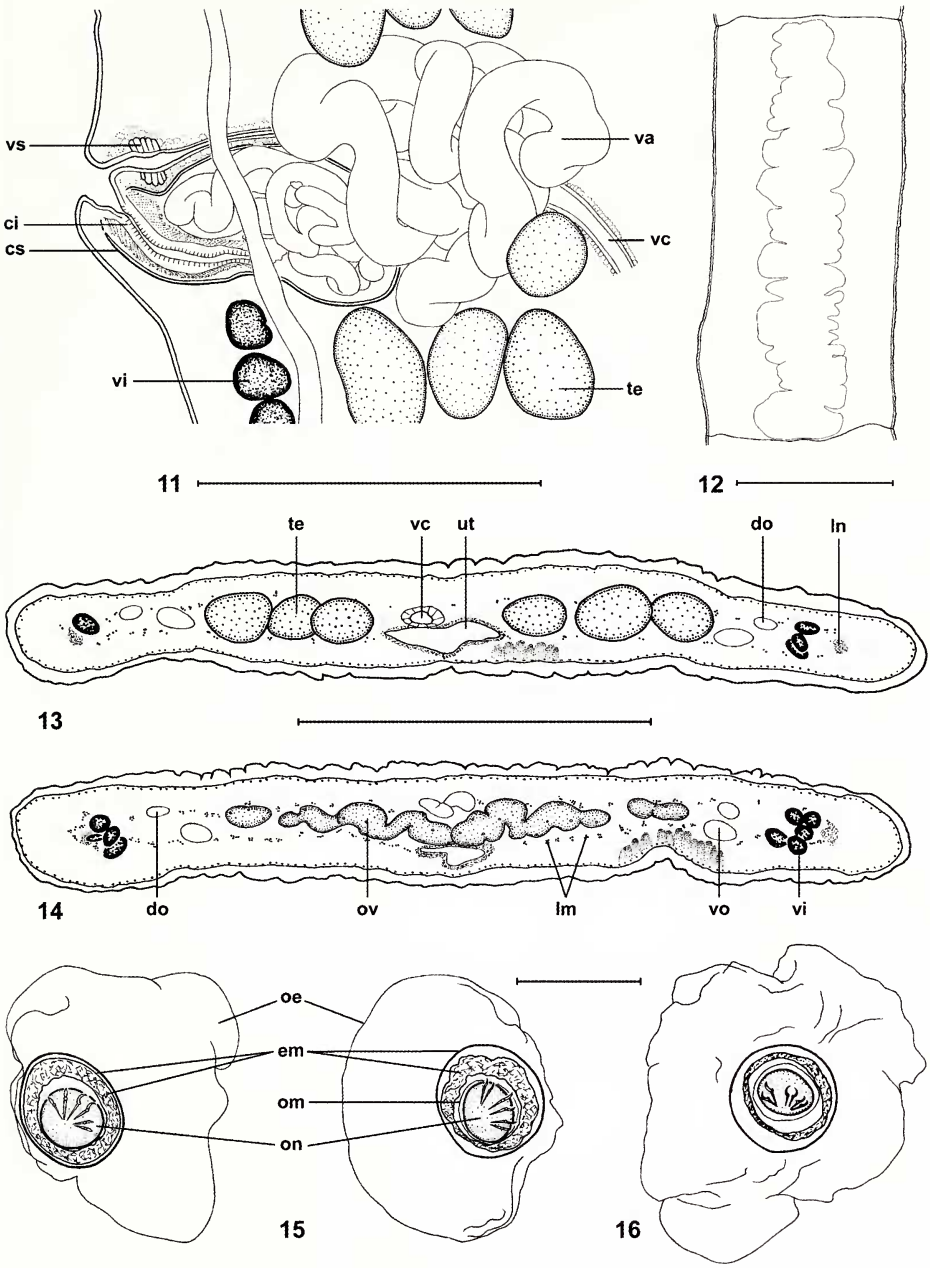
The present species differs from *Pseudocrepidobothrium eirasi*, the only other species known in that genus, by the following characters: absence of appendix at the ventral posterior edge of each side of the proglottis, absence of a polar structure on the egg, number of testes (37-79, x = 55 in *P. ludovici* versus 21-51, x = 32), the disposition of vitelline follicles (more numerous posteriorly in *P. eirasi*) the number of segments (20-36 in *P. ludovici* versus 7-12 for *P. eirasi*) and the shape of the scolex (which is usually rectangular in apical view in *P. ludovici* and square in apical view in *P. eirasi*, see Fig. 2 in the present paper, and Fig. 9 in Rego and Ivanov, 2001).

In a recent paper (de Chambrier *et al.*, 2004a, Fig. 1), the genus *Pseudocrepidobothrium*, including by *P. eirasi* and *Pseudocrepidobothrium* sp. (= *P. ludovici* sp. n.) represented a natural taxon, because the 28S sequences data strongly supported a close relationship between both species. In a forthcoming paper dealing with molecular reconstructions, both species also represent a monophyletic group (unpublished data).



FIGS 7-10. *Pseudocrepidobothrium ludovici* sp. n. from *Phractocephalus hemioliopterus*. (7) MHNG INVE 79281, paratype. Scolex, dorsoventral view. (8) MHNG INVE 22003, holotype. Mature proglottis, ventral view. (9) Mature proglottis, dorsal view; note secondary canals of the ventral osmoregulatory canals. (10) Gravid proglottis, ventral view. *Abbreviations*: do = dorsal osmoregulatory canal; mg = Mehlis gland; ov = ovary; sc = secondary canal; te = testes; ut = uterus; va = vas deferens; vd = vitelloduct; vi = vitellaria; vo = ventral osmoregulatory canal. Scale-bars: 7 = 250 μm; 8-10 = 500 μm.

FIGS 11-16. *Pseudocrepidobothrium ludovici* sp. n. from *Phractocephalus hemioliopterus*. (11) MHNG INVE 79283, paratype. Cirrus-sac and vagina, dorsal view; note the presence of a vaginal sphincter. (12) Schematic view of the uterus in gravid proglottis. (13) MHNG INVE 79341, paratype. Pregravid proglottis, transverse section at testes level. (14) MHNG INVE 79341, paratype. Pregravid proglottis, transverse section at ovarian level. (15, 16) Eggs in distilled water, captured with Leica DMLB, showing the bi-layered embryophore. (15) IPCAS C-610,



(Field number Br 649 3/5w). (16) MHNG INVE 22103, (field number Br 445). *Abbreviations*: ci = cirrus; cs = cirrus-sac; do = dorsal osmoregulatory canal; em = embryophore; lm = internal longitudinal musculature; ln = longitudinal lateral nerves; oe = outer envelope; om = onco - spheric membrane; on = oncosphere; ov = ovary; te = testes; ut = uterus; va = vas deferens; vc = vaginal canal; vi = vitellaria; vo = ventral osmoregulatory canal; vs = vaginal sphincter. Scale-bars: 12 = 500 μm; 11, 13, 14 = 250 μm; 15, 16 = 20 μm.

DISCUSSION

The catfish *Phractocephalus hemioliopterus* from the Amazon River basin has been found to host the following six tapeworm species: 1) *Zygobothrium megacephalum* Diesing, 1850; 2) *Scholzia emarginata* (Diesing, 1850), [synonyms: *Tetrabothrium emarginatum* Diesing, 1850; *Nomimoscolex emarginatum* (Diesing, 1850) Rego *et al.*, 1999; *Myzophorus pirarara* Woodland, 1935; *Nomimoscolex pirarara* (Woodland, 1935); *Proteocephalus pirarara* (Woodland, 1935) de Chambrier & Vaucher, 1997]; 3) *Proteocephalus hemioliopteri* de Chambrier & Vaucher, 1997 [synonyms: *Myzophorus woodlandi* Rego, 1984; *Nomimoscolex woodlandi* (Rego, 1984) Rego & Pavanelli, 1992]; 4) *Pseudocrepidobothrium eirasi* (Rego & de Chambrier, 1995); 5) *Ephedrocephalus microcephalus* Diesing, 1850; 6) *Chambriella* sp. (Diesing, 1850; Woodland, 1935; Rego, 1984; Rego & Pavanelli, 1992; Rego & de Chambrier, 1995; de Chambrier & Vaucher, 1997, 1999; Rego *et al.*, 1999; de Chambrier *et al.*, 2004b; de Chambrier *et al.*, 2006). *Pseudocrepidobothrium ludovici* sp. n. represents the seventh proteocephalidean species parasitizing the intestines of *P. hemioliopterus*.

The uterus of *Pseudocrepidobothrium* occupies the whole length of the proglottis and reaches beyond the ovary, which is unusual among Proteocephalidea. All *Rudolphiella* species [*R. lobosa* (Riggenbach, 1895), *R. piranabu* (Woodland, 1934), *R. myoides* (Woodland, 1934), *R. szidati* Gil de Pertierra & de Chambrier, 2000, *R. piracatinga* (Woodland, 1935)], and some other taxa such as *Brooksiella praeputialis* (Rego, Dos Santos & Silva, 1974), *Proteocephalus sophiae* de Chambrier & Rego, 1994 or *Cairaella henrii* Coquille & de Chambrier, 2008 possess an uterus occupying the whole length of the proglottis (Riggenbach, 1896; Woodland, 1934, 1935; Rego, Santos & Silva, 1974; de Chambrier & Rego, 1994; Gil de Pertierra & de Chambrier, 2000; Coquille & de Chambrier, 2008). In comparison with the about 400 known proteocephalidean species, which possess a uterus rarely overlapping the ovary isthmus posteriorly (Freze, 1965; Schmidt, 1986; Rego *et al.,* 1999; de Chambrier et Vaucher, 1999; de Chambrier *et al.*, 2004b), this character is interesting as discriminant character.

In one *Phractocephalus hemioliopterus* measuring 108 cm in length (field number Br 649), we divided the intestine into five parts and fixed each part separately. We found a total of 12228 proteocephalidean cestodes: 84 *Chambriella* sp., 1 *Ephedrocephalus microcephalus*, 15 *Proteocephalus hemioliopteri*, 10641 *Pseudocrepidobothrium eirasi*, 1100 *P. ludovici* sp. n., 383 *Scholzia emarginata*, and 4 *Zygobothrium megacephalum* in the entire intestine. We sorted each part of the intestine separately and calculated the percentage of each species in each region (see Table 1). *P. eirasi* represented the most abundant species in this individual host with 87% of all tapeworms, followed by *P. ludovici* with 9%. It was observed that 97.6% of *P. eirasi* specimens were situated in the first two fifth of the intestine, and 74.1% of *P. ludovici* tapeworms occupied the same location. *Zygobothrium megacephalum* and *Ephedrocephalus microcephalus* were situated in the last three region of the intestine.

We found neither *P. eirasi* nor *P. ludovici* in the 11 *Phractocephalus hemioliopterus* dissected at Iquitos, Peru. This suggests that Peru might be out of the distribution of these two parasites. If we compare the prevalence of *P. eirasi* and *P. ludovici* in Brazil (55% and 41%, respectively), it is surprising not to observe these parasites in

TABLE 1. Species recovered — Region of the intestine (from the anterior to the posterior end)

Phractocephalus hemioliopterus	I	II	III	IV	V	Total number (Int)
Pseudocrepidobothrium ludovici	295 (26.8%)	520 (47.3%)	232 (21.1%)	41 (3.7%)	12 (1.1 %)	1100 (9.0%)
Pseudocrepidobothrium eirasi	7058 (66.3%)	3325 (31.2%)	225 (2.1%)	27 (0.3%)	6 (0.1 %)	10641 (87%)
Scholzia emarginata	310 (80.9%)	73 (19.1%)				383 (3.1%)
Chambriella sp.	33 (39.3%)	51 (60.7%)				84 (0.7%)
Proteocephalus hemioliopteri	13 (86.7%)	1 (6.7%)	1 (6.7%)			15 (0.1%)
Zygobothrium megacephalum			3 (75%)	1 (25%)		4 (0.03%)
Ephedrocephalus microcephalus				1 (100%)		1 (0.008%)
Total number of worms (Int)						12228 (100%)

the same river, just slightly upstream from the type locality. These variations in local prevalence might therefore depend on unknown biotic or abiotic factors.

P. ludovici is the seventh species and the sixth genus of Proteocephalidea described from *P. hemioliopterus*. It is interesting to note that some Amazonian siluriforms also host a huge diversity of proteocephalidean genera: *Paulicea luetkeni* (= *Zungaro zungaro*) hosts seven genera representing seven species, *Pseudoplatystoma fasciatum* (= *P. punctifer*) can be infected with six genera representing seven species and *Brachyplatystoma flavicans* (= *B. rousseauxi*) harbours four genera representing five species (Rego *et al.*, 1999).

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